High-Pass DNA Size Selection for Recovery of a Highly Degraded Sample for Oxford Nanopore Long-Read Sequencing

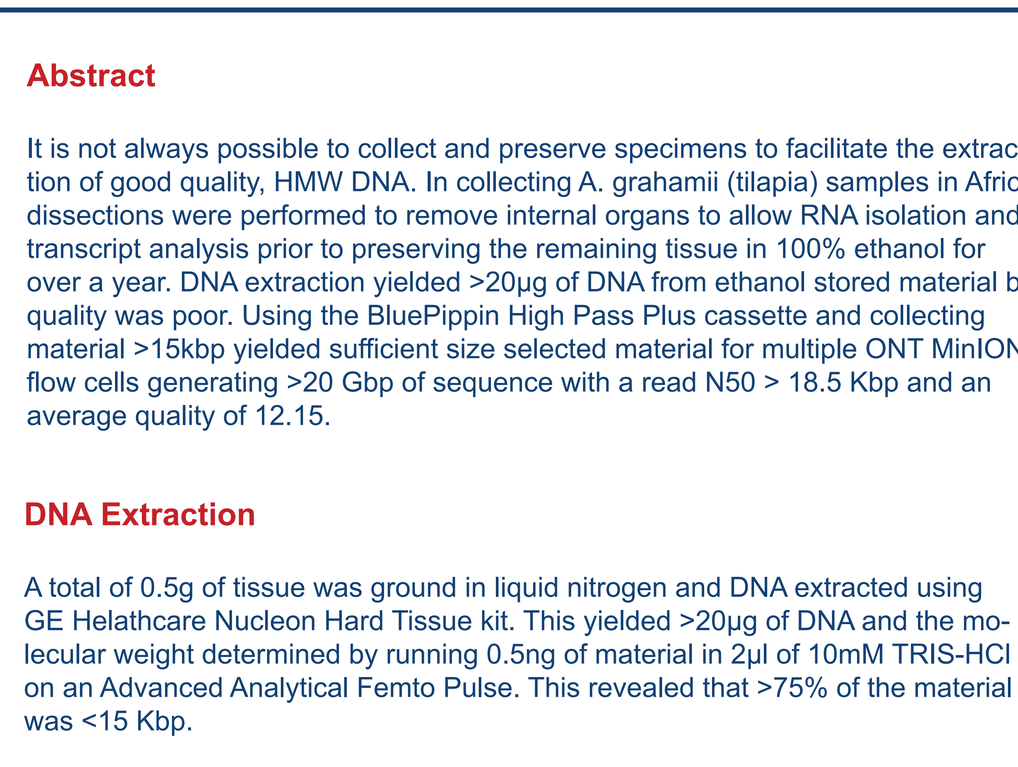
Darren Heavens, Earlham Institute

Abstract

It is not always possible to collect and preserve specimens to facilitate the extraction of good quality, HMW DNA. In collecting A. grahamii (tilapia) samples in Africa, dissections were performed to remove internal organs to allow RNA isolation and transcript analysis prior to preserving the remaining tissue in 100% ethanol for over a year. DNA extraction yielded >20µg of DNA from ethanol stored material but quality was poor. Using the BluePippin High Pass Plus cassette and collecting material >15kbp yielded sufficient size selected material for multiple ONT MinION flow cells generating >20 Gbp of sequence with a read N50 > 18.5 Kbp and an average quality of 12.15.

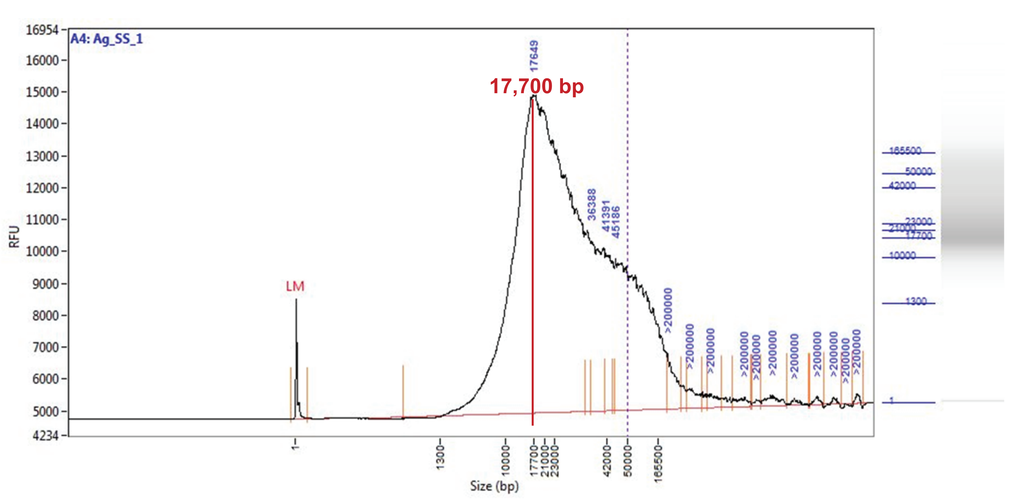
DNA Extraction

A total of 0.5g of tissue was ground in liquid nitrogen and DNA extracted using GE Helathcare Nucleon Hard Tissue kit. This yielded >20µg of DNA and the molecular weight determined by running 0.5ng of material in 2µl of 10mM TRIS-HCl on an Advanced Analytical Femto Pulse. This revealed that >75% of the material was <15 Kbp.



HIgh Pass Plus DNA Size Selection

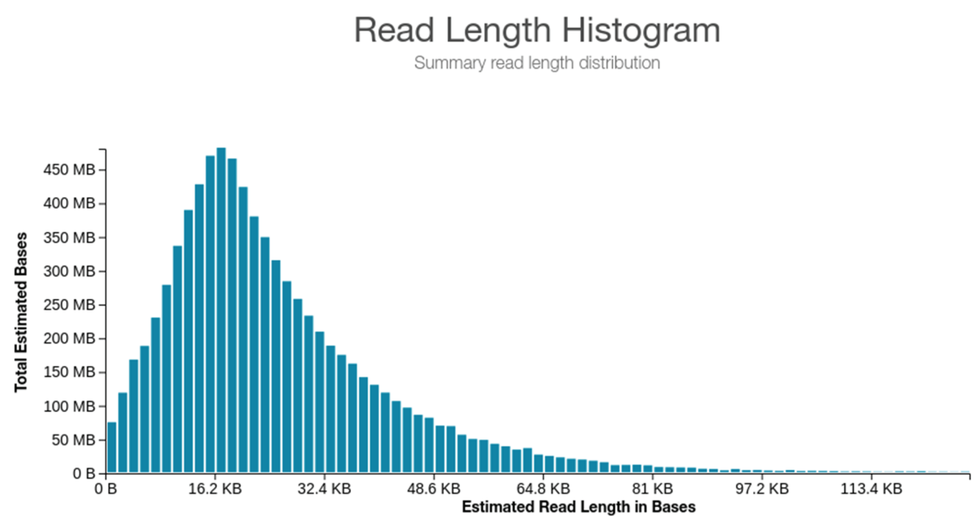
The >15kb size selection protocol was run on three lanes (6 µg / lane). Recovered eluants were pooled and concentrated using Kapa Pure beads (Roche Sequencing solutions). Approx. 2 µg of DNA was recovered. Fragment analysis indicated a more favourable size profile with an expected cut-off at 15 kb. The size selection run time was 3 hours 15 minutes. Based on the estimation of the amount of material >15 Kbp in the extracted DNA the recovery from the cassette represented 48% of the material present.



Fragment Size Distribution After >15 Kb DNA Size Selection and Bead Clean-up

ONT Library Construction and Sequencing

Libraries were constructed using the ONT SQK-LSK109 genomic DNA kit with no fragmentation. Sufficient library molecules were generated for two MinION 106 flow cells and combined these generated >20 Gbp of sequence (circa 20x genome coverage).



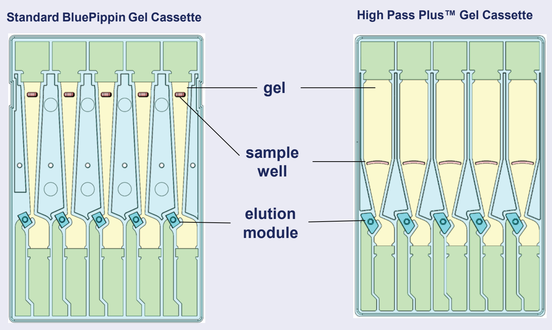
Flow cell run metrics

NumReads: 915879
TotalSum: 10426384900
MeanLength: 11384.02
Shortest: 5
Longest: 210418
N50Length: 18782
N50Count: 179758
N90Length: 7312
N90Count: 507505
averageQScore: 12.15

BluePippin HIgh Pass Plus DNA Size Selection

Sage Science has made modifications to the BluePippin gel cassette design for optimizing performance specifically for high pass DNA size selection. The goal of the effort was to improve performance in the following areas:

- Faster Run Times
- Higher Sample Yield



- Shortened gel column: Provides shorter run time.
- Exaggerated taper: Increases sample loading capacity.
- Thinner sample well/wider column: Improves resolution.
- Enlarged elution well: Increases sample recovery.

sage science

Author Contact: Darren.Heavens@earlham.ac.uk